# Qualitative Analysis of Piecewise-Affine Models of Genetic Regulatory Networks

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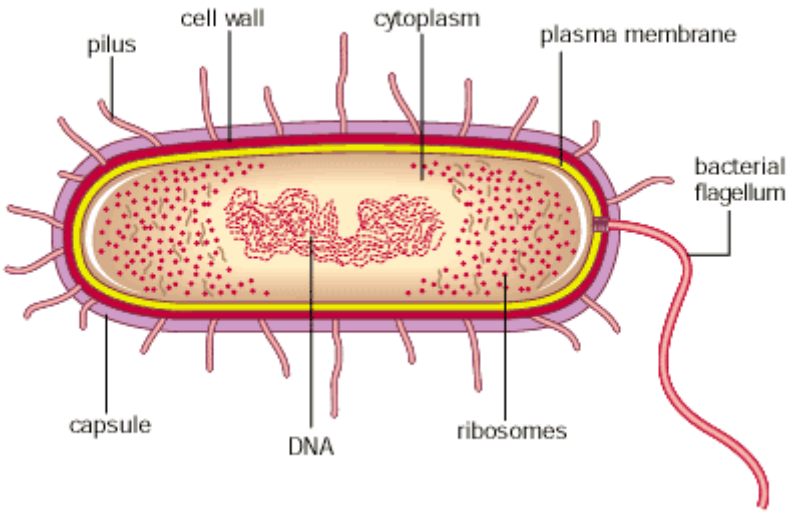
# Overview

1. Genetic regulatory networks
2. Modeling of genetic regulatory networks: objective and constraints
3. Piecewise-affine models of genetic regulatory networks
4. Qualitative analysis and verification of piecewise-affine models
5. Genetic Network Analyzer (GNA)
6. Conclusions and perspectives

# Bacterial cell and proteins

- Proteins are building blocks of cell:
  - Transport of nutrients and waste products across cell membrane
  - Extraction of energy from nutrients
  - Control of growth and division
  - Adaptation to external perturbations

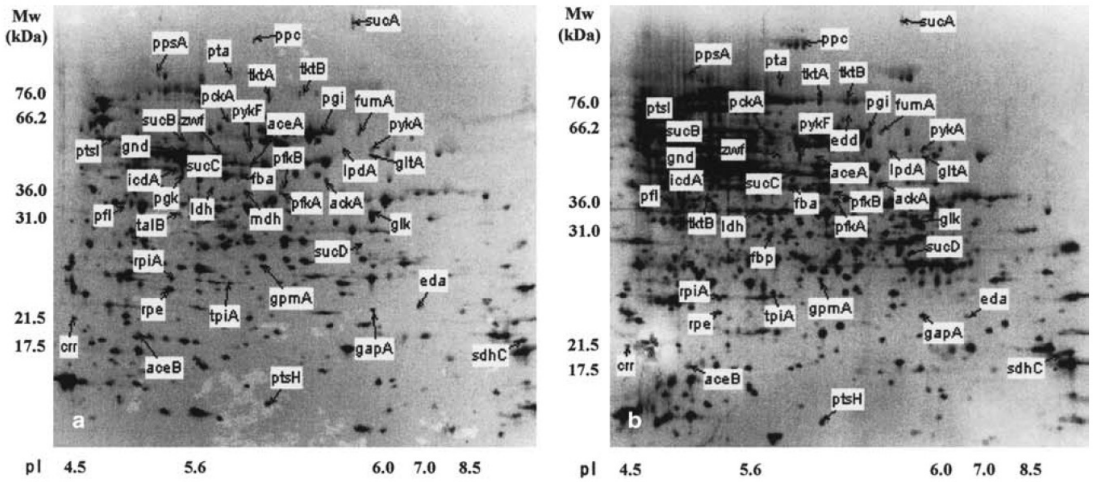


# Variation in protein levels

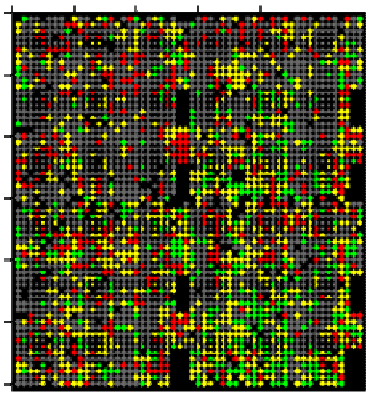
- Protein levels in cell are adjusted to specific environmental conditions

Peng, Shimizu (2003),
*App. Microbiol. Biotechnol.*, 61:163-178

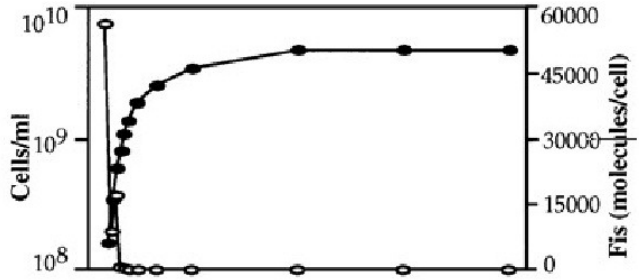
2D gels




DNA
microarrays

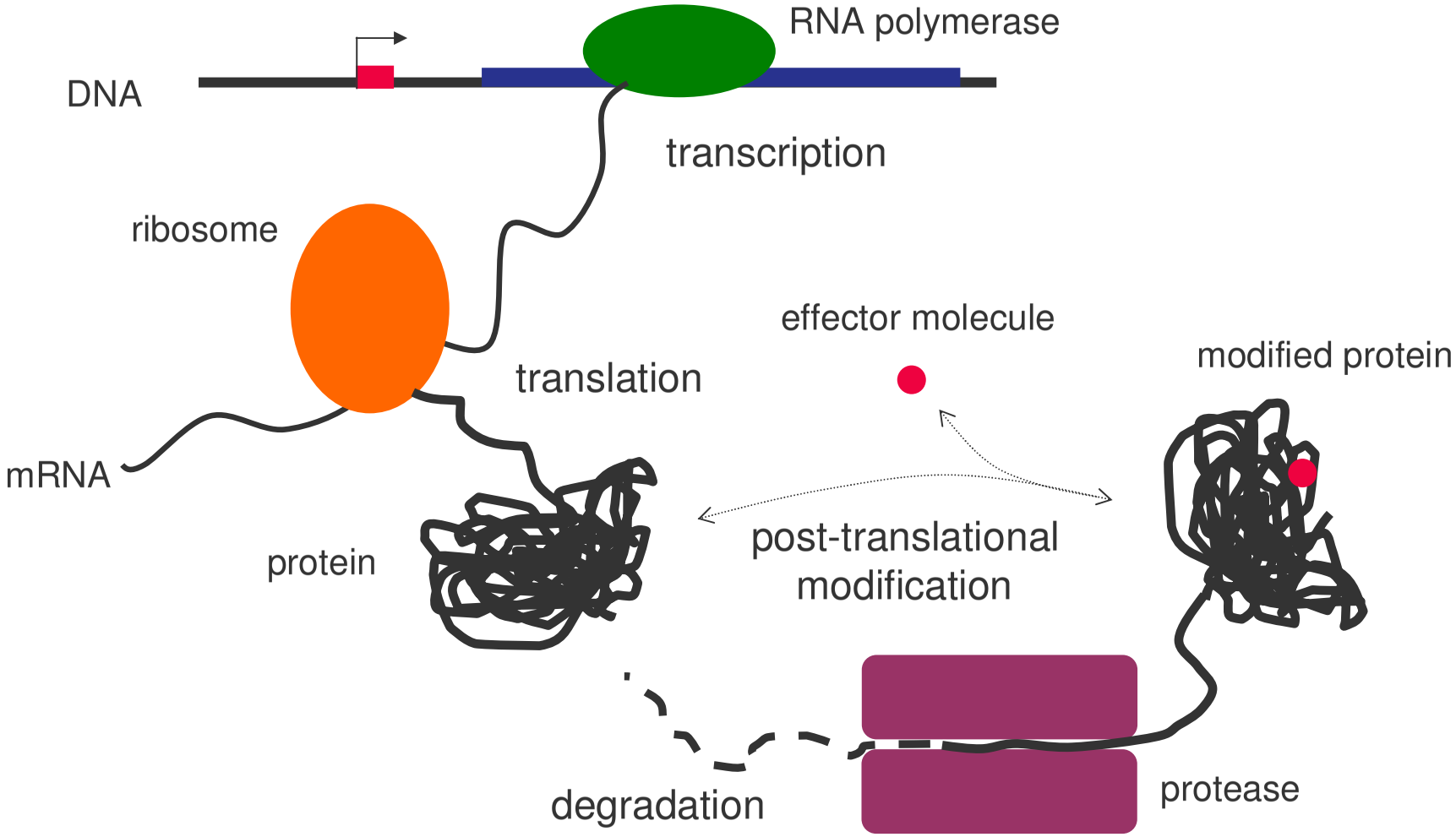


Western blots

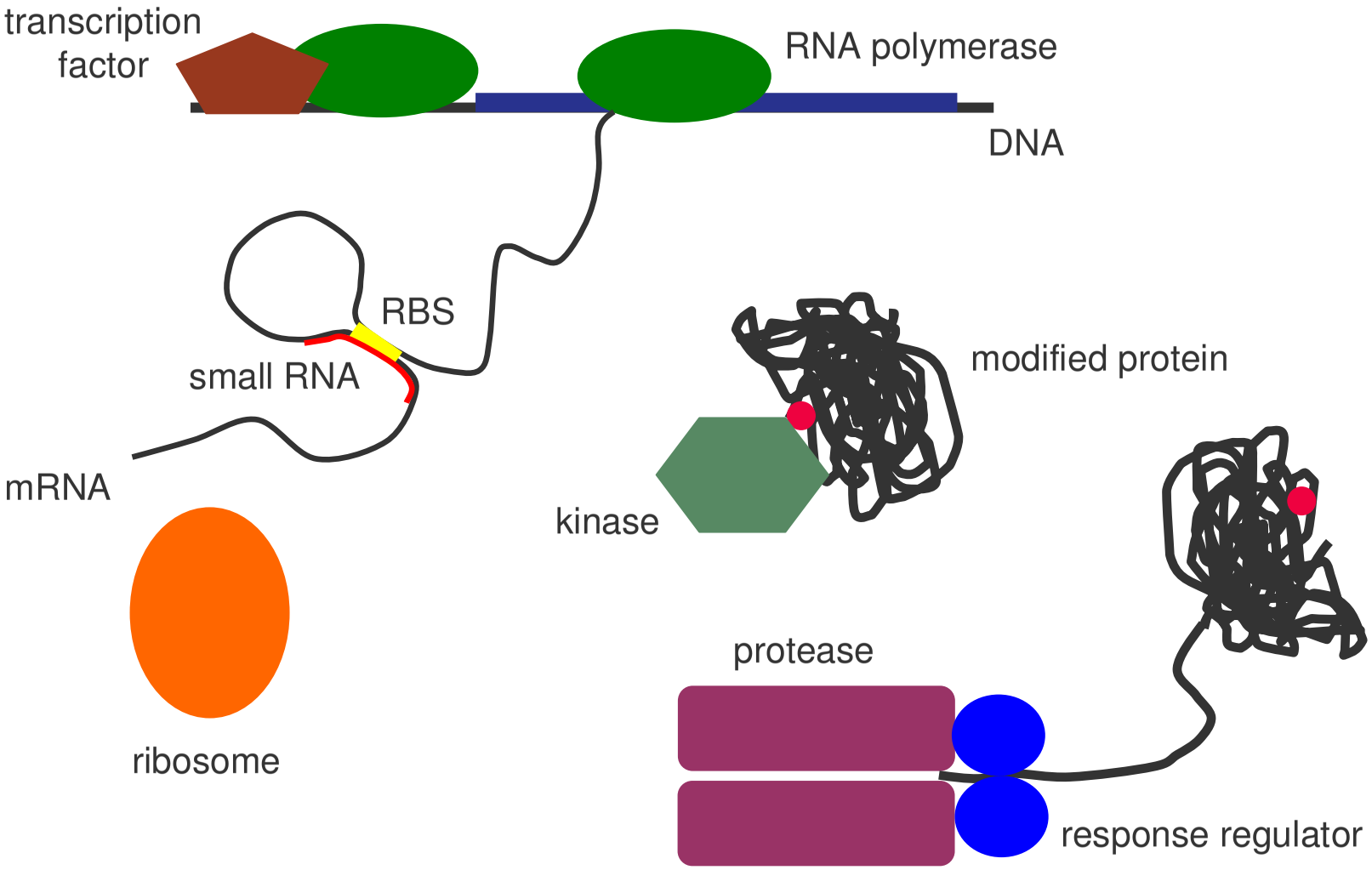



Ali Azam *et al.* (1999), *J. Bacteriol.*, 181(20):6361-6370

# Synthesis and degradation of proteins



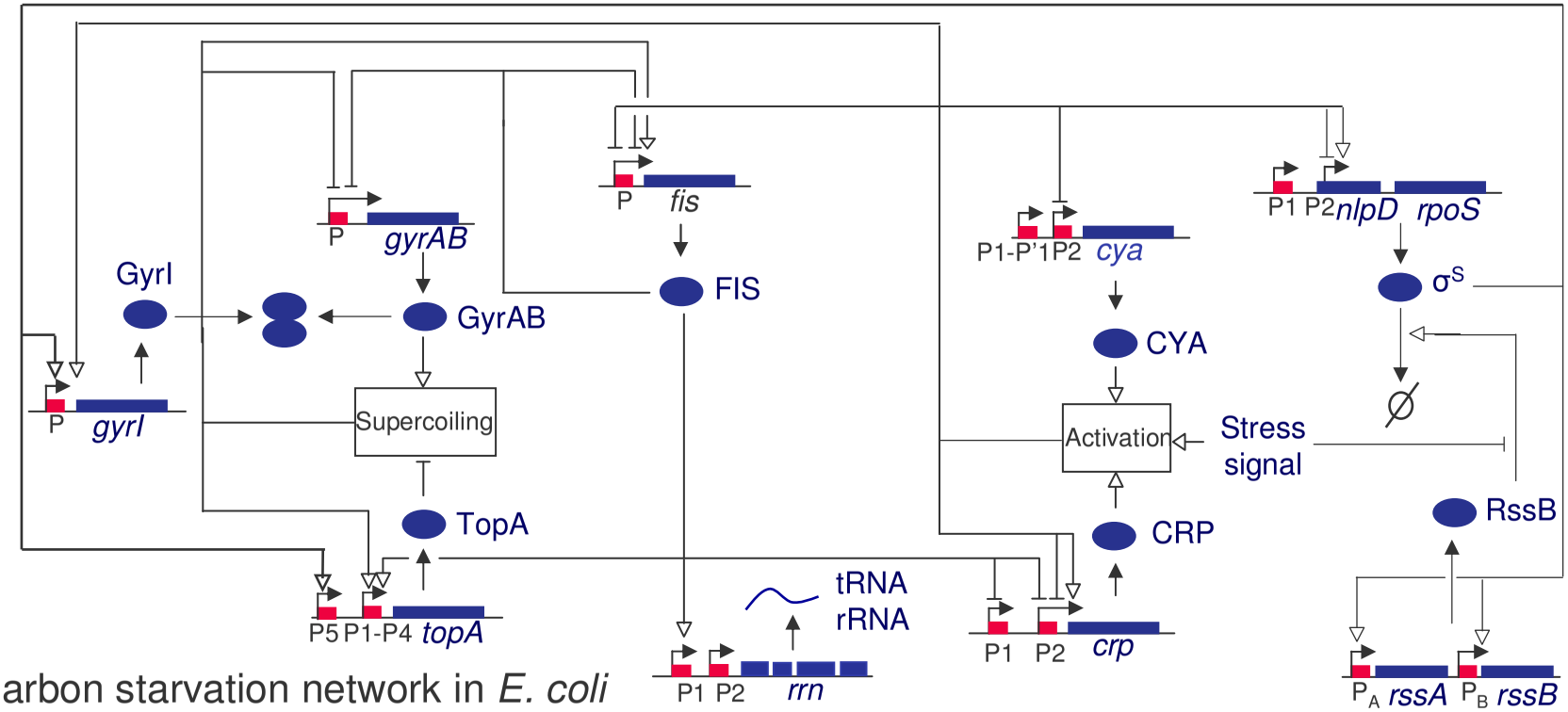
# Regulation of synthesis and degradation



# Genetic regulatory networks

- Control of protein synthesis and degradation gives rise to **genetic regulatory networks**

  Networks of genes, RNAs, proteins, metabolites, and their interactions




Carbon starvation network in *E. coli*

# Analysis of genetic regulatory networks

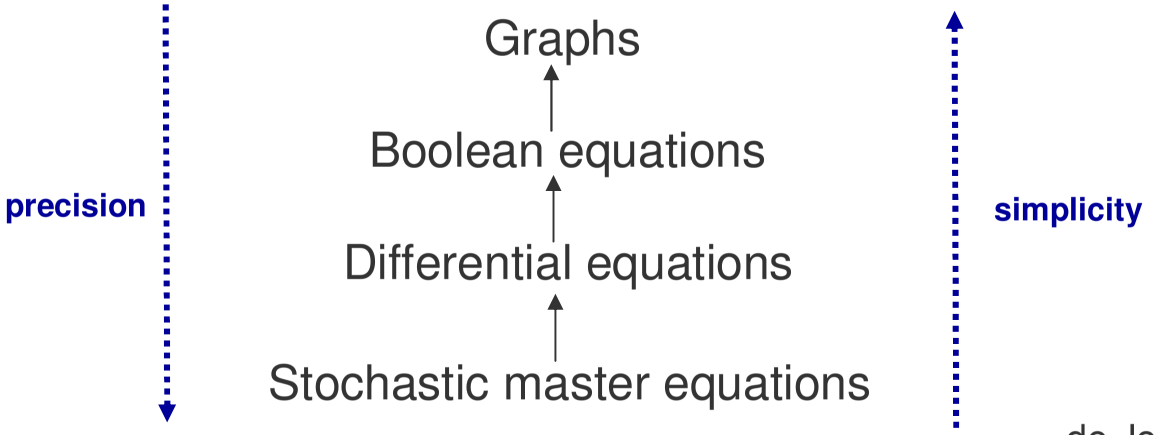
- Abundant knowledge on components and interactions of genetic regulatory networks
  - Scientific knowledge bases and databases
  - Bibliographic databases
- Currently no understanding of how global dynamics emerges from local interactions between components
  - Response of cell to external stress
  - Differentiation of cell during development
- Shift from **structure** to **dynamics** of networks

  « functional genomics », « integrative biology », « systems biology », …

Kitano (2002), *Science*, 295(5560):564

# Mathematical methods and computer tools

- **Modeling** and **simulation** indispensable for dynamic analysis of genetic regulatory networks:
  - Understanding role of individual components and interactions
  - Suggesting missing components and interactions
- Variety of modeling formalisms exist, describing system on different levels of detail




de Jong (2002), *J. Comput. Biol.*, 9(1): 69-105

# Constraints on modeling and simulation

- Current **constraints** on modeling and simulation:
  - Knowledge on molecular mechanisms rare
  - Quantitative information on kinetic parameters and molecular concentrations absent
- Possible strategies to overcome the constraints
  - Parameter estimation from experimental data
  - Parameter sensitivity analysis
  - Model simplifications
- Intuition: essential properties of system dynamics **robust** against moderate changes in kinetic parameters and rate laws

Stelling *et al.* (2004), *Cell*, 118(6):675-86

# Qualitative modeling and simulation

- **Qualitative** modeling and simulation of large and complex genetic regulatory networks using **simplified** models

  de Jong, Gouzé *et al.* (2004), *Bull. Math. Biol.*, 66(2):301-40

  Batt *et al.* (2007), *Automatica*, accepted for publication

- Applications of qualitative simulation:
  - initiation of sporulation in *Bacillus subtilis*

    de Jong, Geiselmann *et al.* (2004), *Bull. Math. Biol.*, 66(2):261-300
  - quorum sensing in *Pseudomonas aeruginosa*

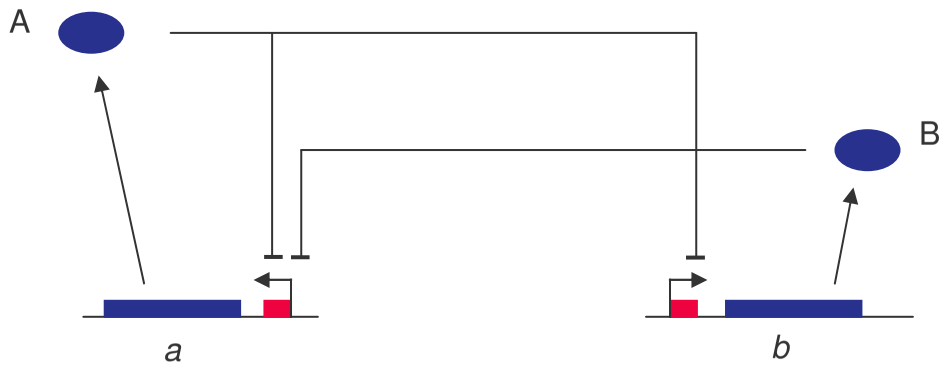
    Viretta and Fussenegger, *Biotechnol. Prog.*, 2004, 20(3):670-678
  - onset of virulence in *Erwinia chrysanthemi*

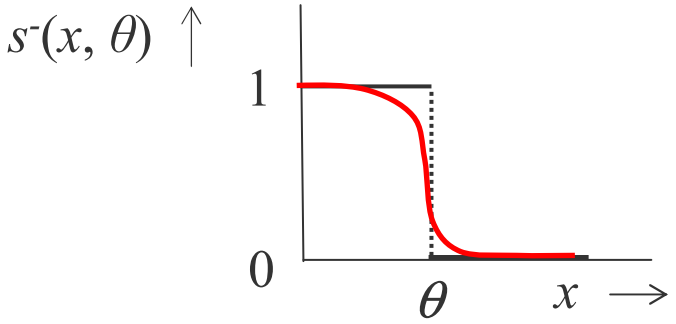
    Sepulchre *et al.*, *J. Theor. Biol.*, 2007, 244(2):239-57

# PA differential equation models

- Genetic networks modeled by class of differential equations using **step functions** to describe regulatory interactions

$$\dot{x}_a = \kappa_a \, s^-(x_a, \theta_{a2}) \, s^-(x_b, \theta_b) - \gamma_a \, x_a$$
$$\dot{x}_b = \kappa_b \, s^-(x_a, \theta_{a1}) - \gamma_b \, x_b$$



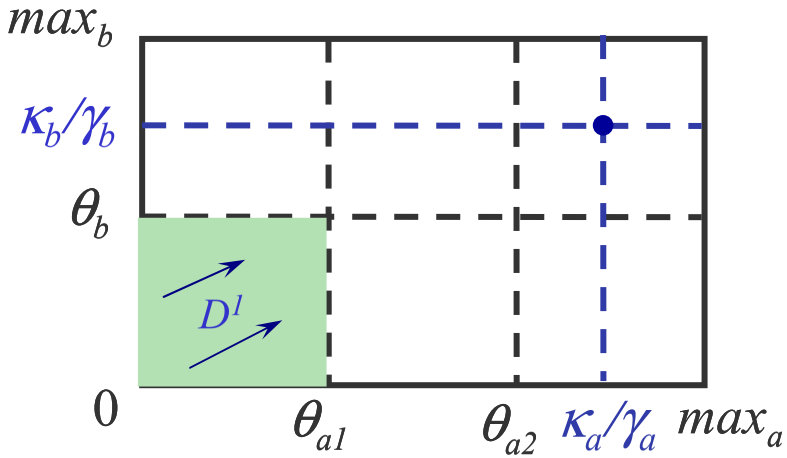

$x$ : protein concentration
$\theta$ : threshold concentration
$\kappa$, $\gamma$: rate constants

- Differential equation models of regulatory networks are **piecewise-affine (PA)**

Glass and Kauffman (1973), *J. Theor. Biol.*, 39(1):103-29

# Mathematical analysis of PA models

- Analysis of dynamics of PA models in phase space




$$\dot{x}_a = \kappa_a - \gamma_a x_a$$
$$\dot{x}_b = \kappa_b - \gamma_b x_b$$

$$\dot{x}_a = \kappa_a \, s^-(x_a, \theta_{a2}) \, s^-(x_b, \theta_b) - \gamma_a x_a$$
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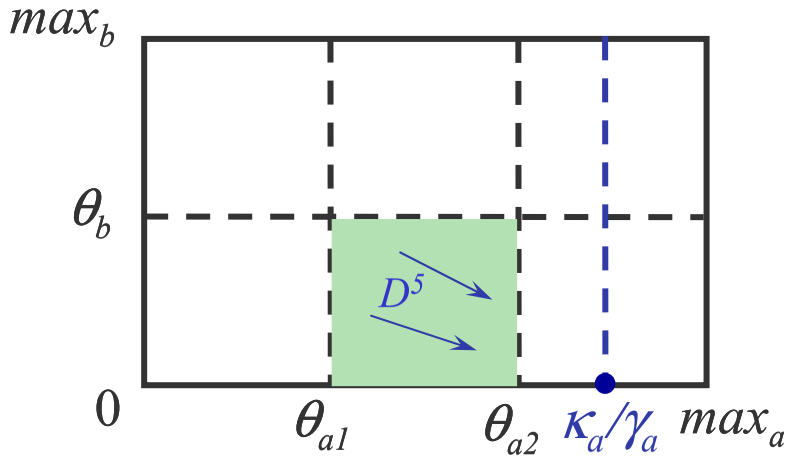


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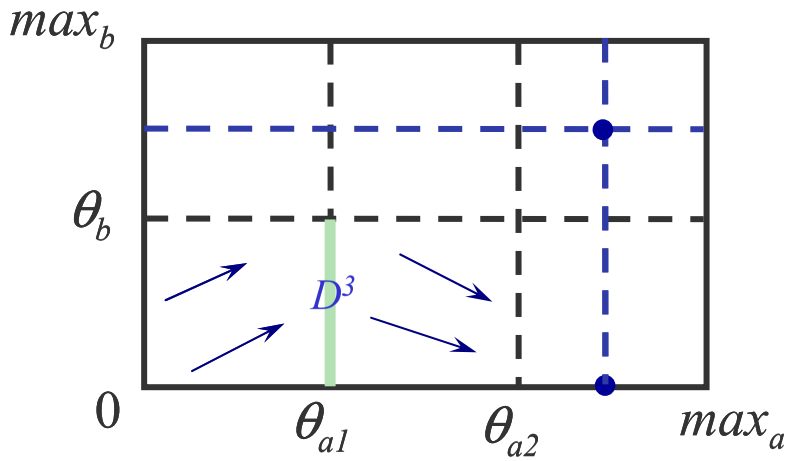


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- Extension of PA differential **equations** to differential **inclusions** using Filippov approach

Gouzé, Sari (2002), *Dyn. Syst.*, 17(4):299-316

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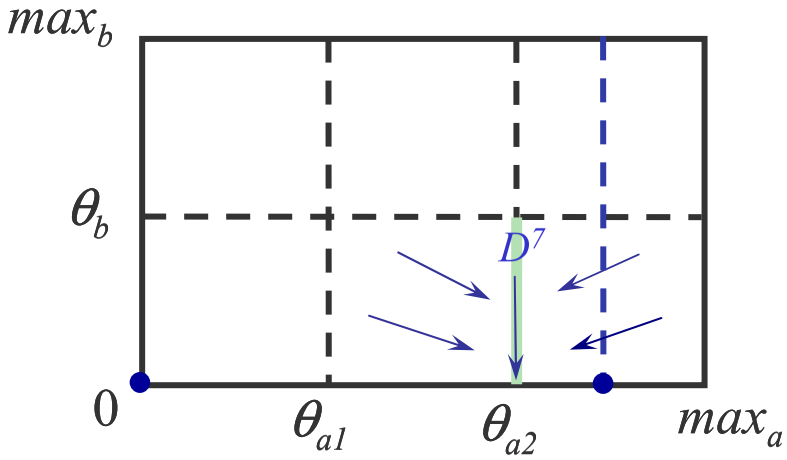


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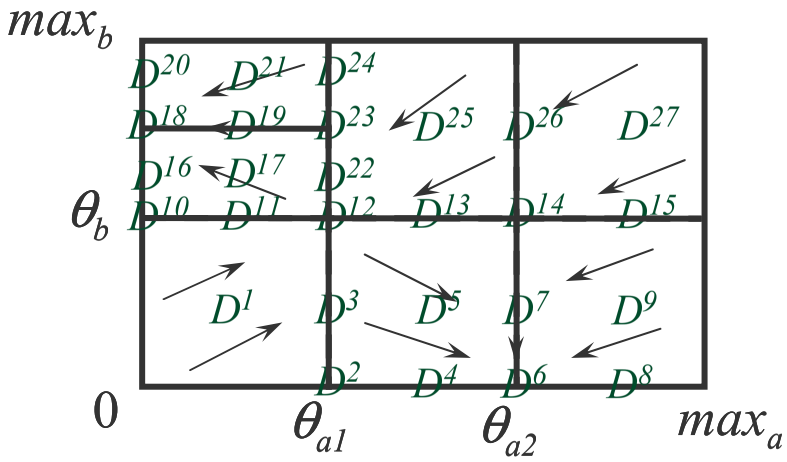
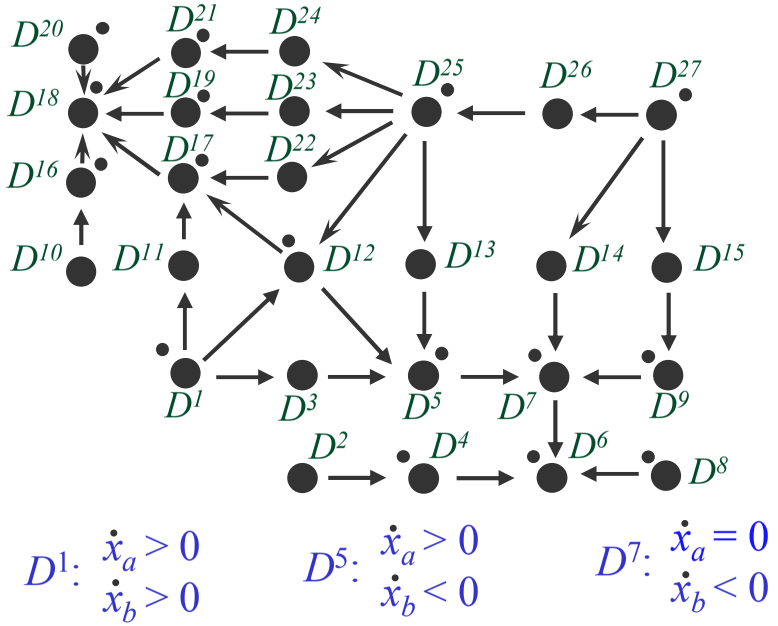
Gouzé, Sari (2002), *Dyn. Syst.*, 17(4):299-316

# Qualitative analysis of network dynamics

- Phase space partition: **unique** derivative sign pattern in regions
- **Qualitative abstraction** yields state transition graph

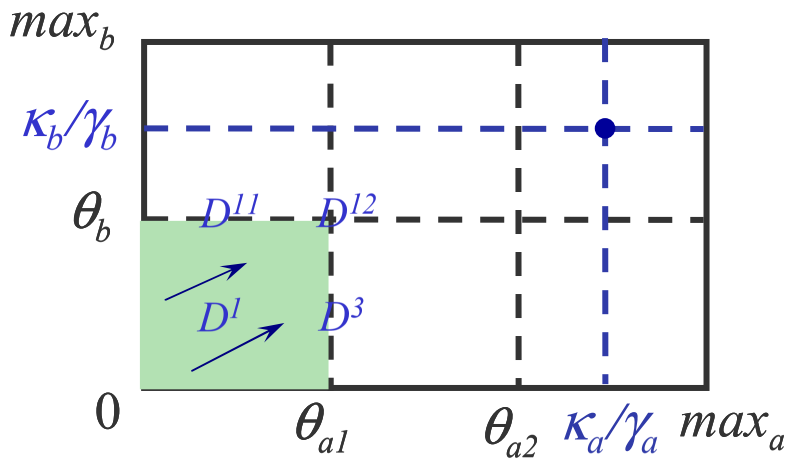
  Shift from continuous to discrete picture of network dynamics

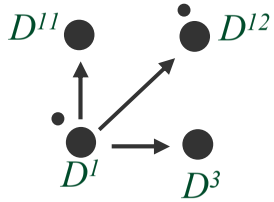


# Qualitative analysis of network dynamics

- State transition graph **invariant** for parameter constraints




$$0 < \theta_{a1} < \theta_{a2} < \kappa_a/\gamma_a < max_a$$

$$0 < \theta_b < \kappa_b/\gamma_b < max_b$$

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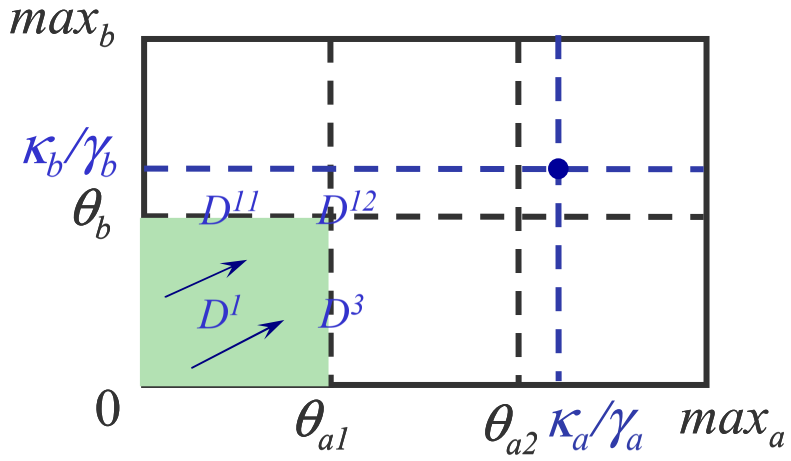


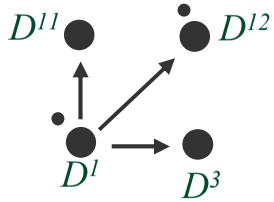


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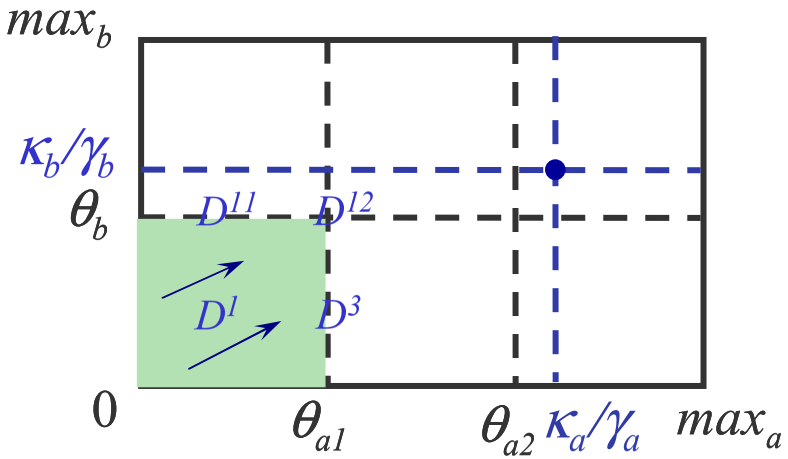


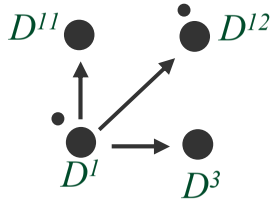


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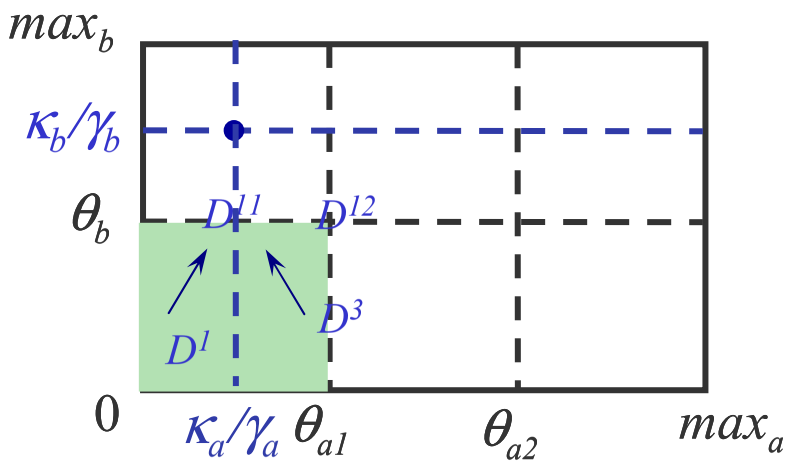


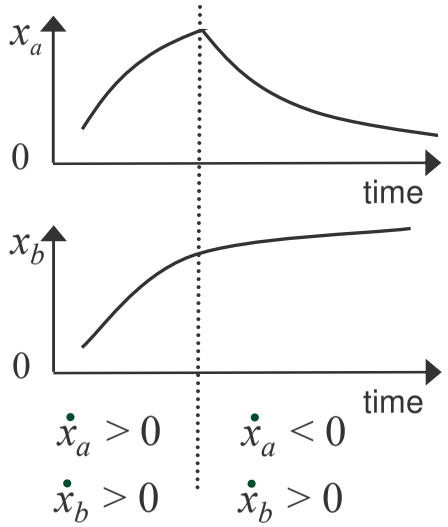


$$0 < \kappa_a/\gamma_a < \theta_{a1} < \theta_{a2} < max_a$$

$$0 < \theta_b < \kappa_b/\gamma_b < max_b$$

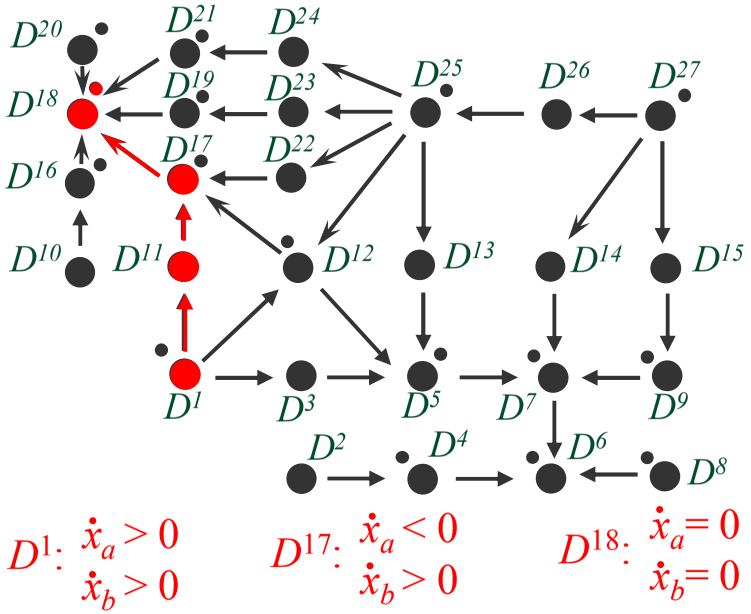
# Validation of qualitative models

- Predictions well adapted to comparison with available experimental data: **changes of derivative sign patterns**




Concistency?

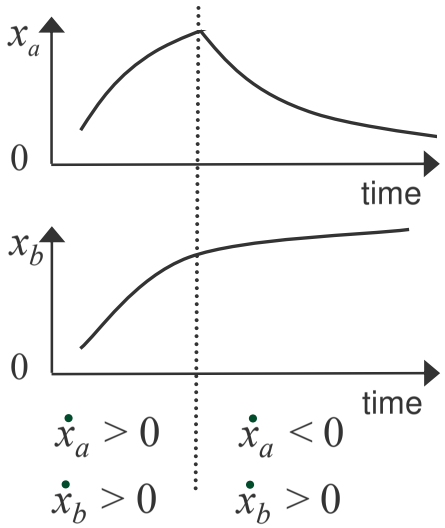
Yes




- **Model validation**: comparison of derivative sign patterns in observed and predicted behaviors
- Need for automated and efficient **tools** for model validation

# Validation using model checking

- Compute state transition graph and express dynamic properties in temporal logic

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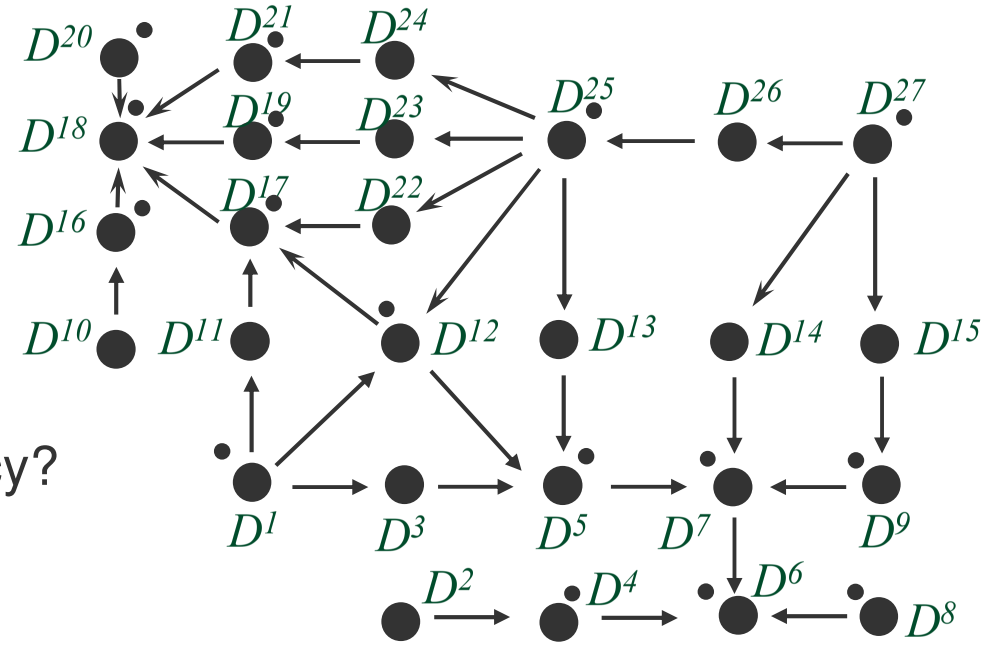


- Use of model checkers to verify whether experimental data and predictions are consistent

Batt *et al.* (2005), *Bioinformatics*, 21(supp. 1): i19-28

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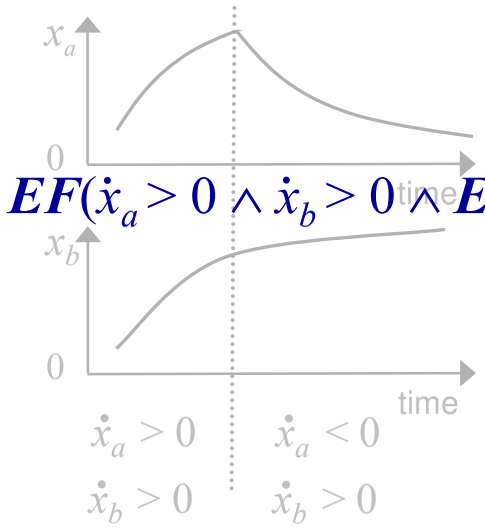
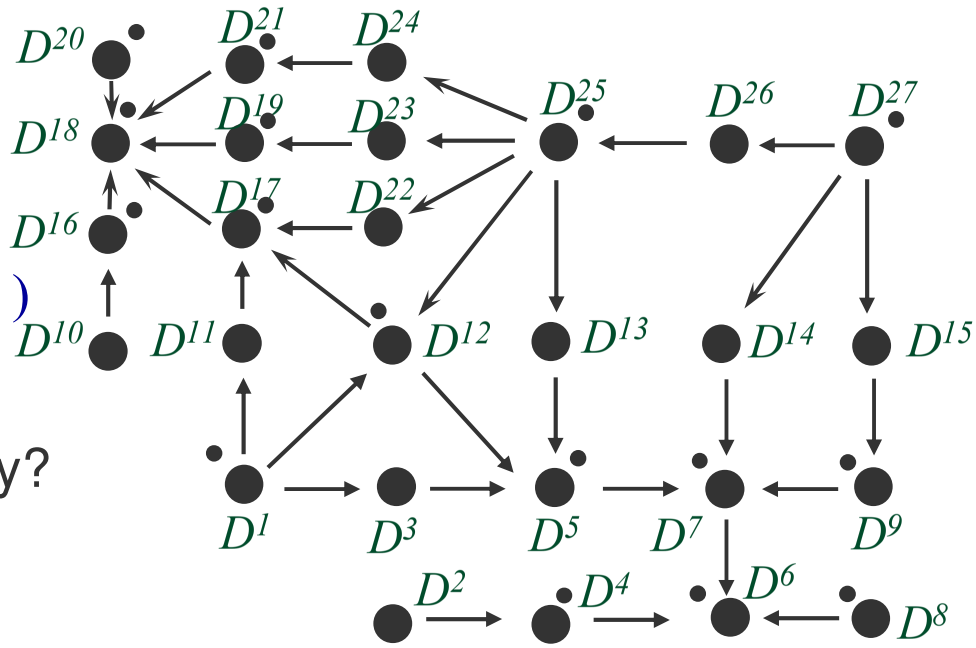



$$EF(\dot{x}_a > 0 \wedge \dot{x}_b > 0 \wedge EF(\dot{x}_a < 0 \wedge \dot{x}_b > 0))$$

Concistency?

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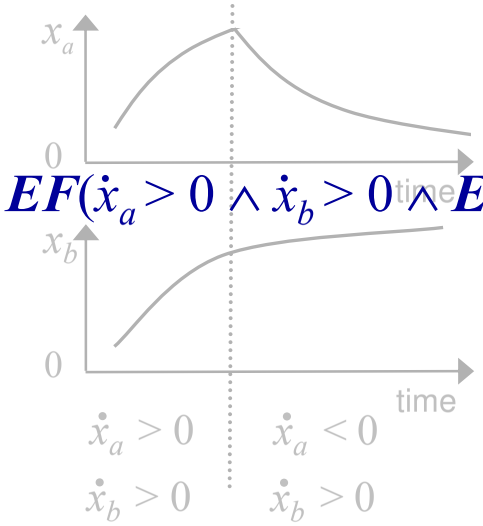


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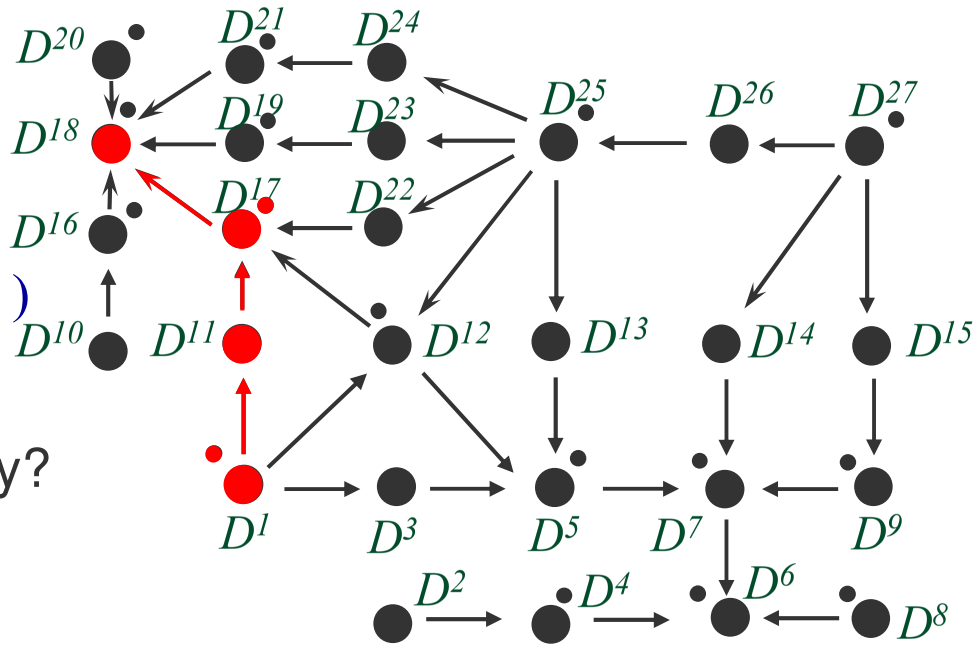


- Use of model checkers to verify whether experimental data and predictions are consistent

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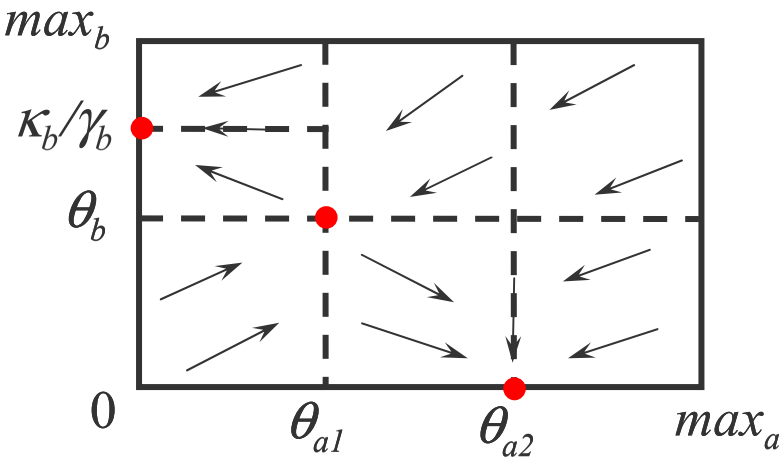
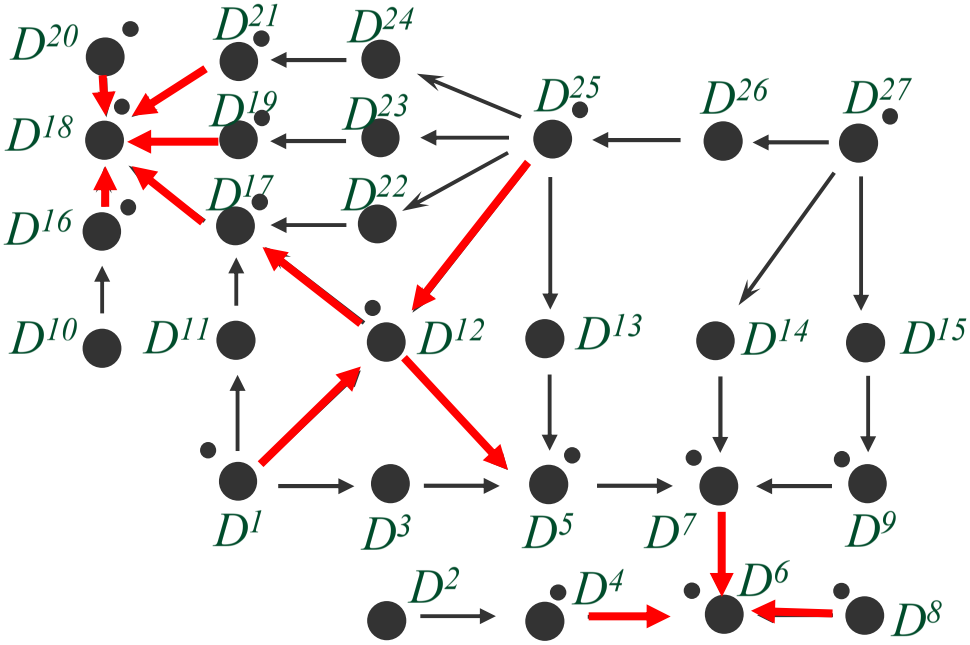
# Analysis of attractors of PA systems

- Search of **attractors** of PA systems in phase space



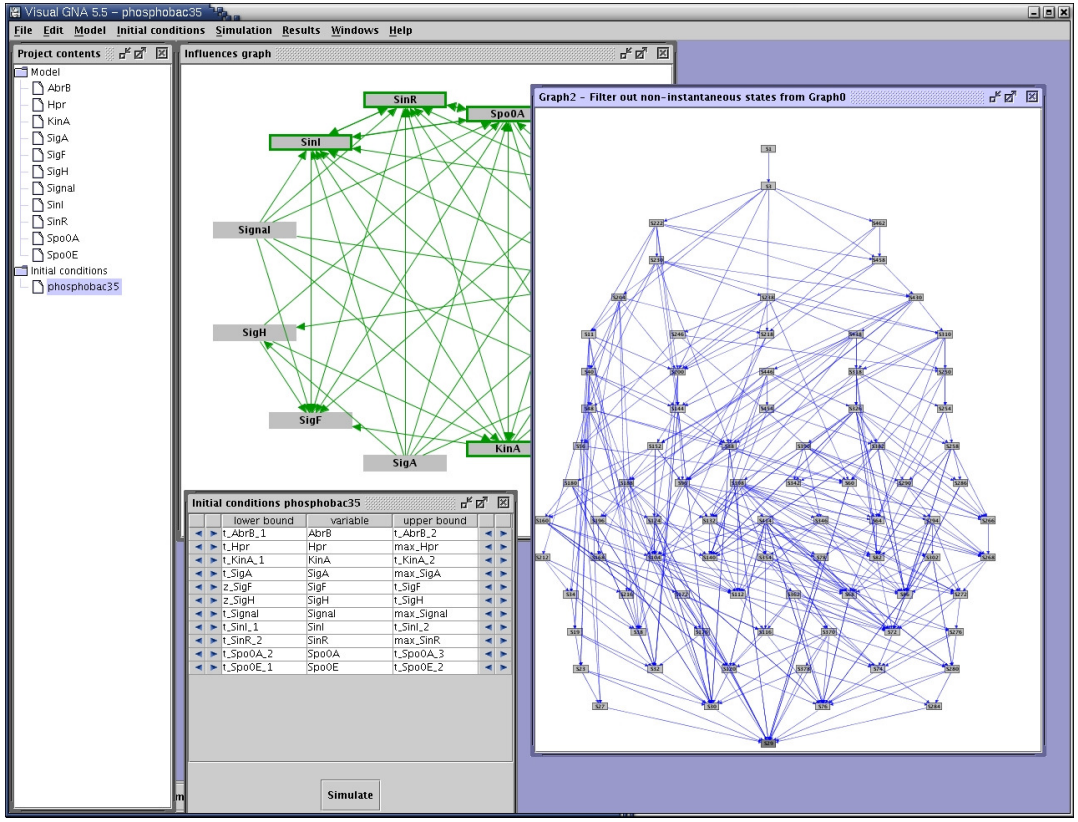

- Analysis of **stability** of attractors, using properties of state transition graph

Casey *et al.* (2006), *J. Math Biol.*, 52(1):27-56

Definition of stability of equilibrium points on surfaces of discontinuity

# Genetic Network Analyzer (GNA)

- Qualitative simulation method implemented in Java: **Genetic Network Analyzer (GNA)**




Distribution by Genostar SA


de Jong *et al.* (2003), *Bioinformatics*, 19(3):336-44

Batt *et al.* (2005), *Bioinformatics*, 21(supp. 1): i19-28

**http://www-helix.inrialpes.fr/gna**

# Perspectives

- Inference of regulatory networks from gene expression data

  Use of hybrid system identification methods adapted to PA models

  Drulhe *et al.* (2006), *Hybrid Systems: Computation and Control*, LNCS 3927, 184-99

- Composite models of metabolic and genetic regulatory networks

  Generalization of qualitative analysis to broader classes of PA models

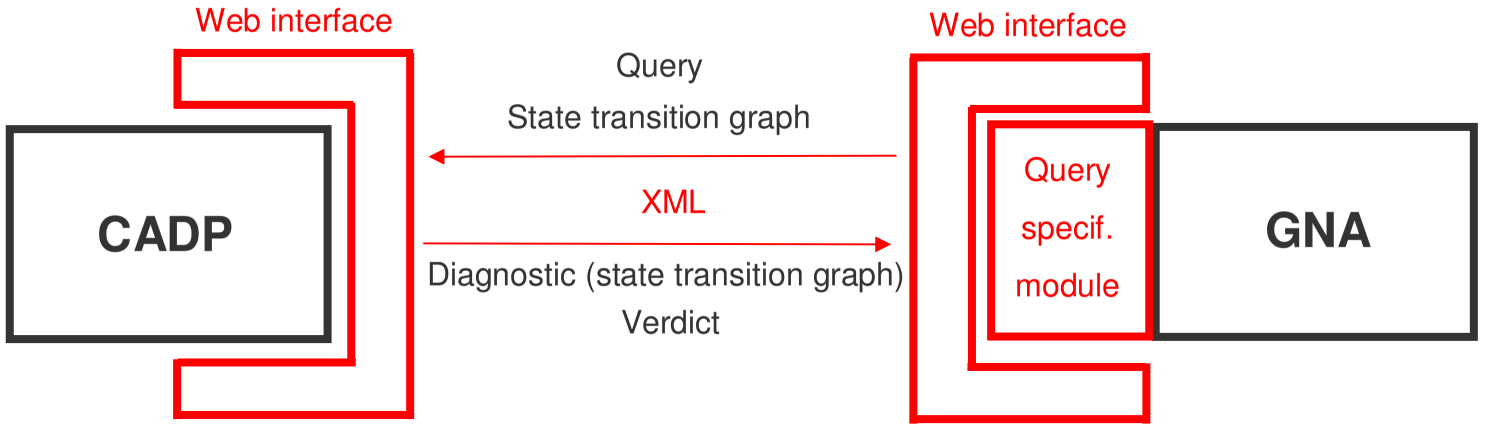
  Musters *et al.* (2007), *Hybrid Systems: Computation and Control*, LNCS 4416, 727-730

- Integrated tools for model checking and qualitative analysis using high-level specification languages

  Prerequisite for further upscaling

# Future coupling of GNA to model checker

- Integration of GNA and formal verification and model checking tools by means of web interface
- CADP: Construction and Analysis of Distributed Processes



# Conclusions

- Understanding of functioning and development of living organisms requires **analysis of genetic regulatory networks**

  From structure to behavior of networks

- Need for **mathematical methods** and **computer tools well-adapted** to **available** experimental data

  Coarse-grained models and qualitative analysis of dynamics

- **Biological relevance** attained through **integration** of modeling and experiments

  **Models guide experiments, and experiments stimulate models**

# Contributors and sponsors

Grégory Batt, Université Joseph Fourier, Grenoble

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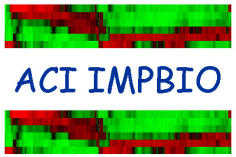
Michel Page, INRIA Rhône-Alpes/Université Pierre Mendès France, Grenoble

Delphine Ropers, INRIA Rhône-Alpes

Tewfik Sari, Université de Haute Alsace, Mulhouse




Ministère de la Recherche, IMPBIO program

European Commission, FP6, NEST program


INRIA, ARC program


Agence Nationale de la Recherche, BioSys program